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UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010

PHASE 8
DERMAL PENETRATION OF THE RADIOLABELED REPELLENT
N,N-DIETHYL-M-TOLUAMIDE (M-DET)
STUDY NO. 75-51-0034-81
JANUARY 1979 - JUNE 1980

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The dermal penetration of ^{14}C-labeled m-Det was measured in rats, rabbits, and dogs. Absorption was quantitated by monitoring radioactivity in excreta for 7 days and tissues at necropsy. Pregnant rabbits were repeatedly applied with the repellent and fetuses monitored for radioactivity at term. It is concluded that topically applied m-Det should not present a dermatoxic hazard to man and absorption would be expected to be less than 10 percent of the applied dose. No affinity for specific tissue binding of the repellent has been demonstrated in these tests.

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

Mr. Snodgrass/lm/AUTOVON
584-3980

5 DEC 1980

HSE-LT/WP

SUBJECT: Phase 8, Dermal Penetration of Radiolabeled Repellent N,N-Diethyl-m-toluamide (m-Det), Study No. 75-51-0034-81, January 1979 - June 1980

Executive Secretary
Armed Forces Pest Management Board
Forest Glen Section
Walter Reed Army Medical Center
Washington, DC 20012

A summary of the pertinent findings of the inclosed report follows:

a. Radiolabeled ^{14}C-labeled N,N-Diethyl-m-toluamide (m-Det) was evaluated for skin penetration in rats, rabbits, and dogs. Absorption was quantitated by measuring radioactivity in urine for 7 days following a single dermal application. Maximum dermal penetration of the repellent occurred within 24 hours and was nearly complete after 3 days. Analysis of tissue specimens monitored at 7 days indicated the absence of significant radioactivity.

b. A concurrent study assessed absorption of m-Det in pregnant rabbits following repeated dermal applications at dose levels comparable to those used in man. No evidence for bioaccumulation of the radioactive chemical was observed in the maternal parents nor in fetuses at term.

c. It is concluded that m-Det when applied to the skin should not present a dermatotoxic hazard to man. Based on these and other investigations, absorption of the repellent would be expected to be less than 10 percent of the applied dose.

FOR THE COMMANDER:

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as (5 cy)

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
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HSE-LT/WP

PHASE 8
DERMAL PENETRATION OF THE RADIOLABELED REPELLENT N,N-DIETHYL-M-TOLUAMIDE (M-DET)
STUDY NO. 75-51-0034-81
JANUARY 1979 - JUNE 1980

1. AUTHORITY.

a. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board; and the US Department of Agriculture, Agriculture Research, Science and Education Administrations; titled Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

b. Letter, Armed Forces Pest Control Board, 17 March 1977, subject: Reregistration Data for N,N-Diethyltoluamide Repellent.

2. PURPOSE. This study was designed to quantitate the penetration of radiolabeled (^{14}C) N,N-Diethyl-m-toluamide (m-Det) through the intact skin of rabbits, rats, and dogs. Three aspects of this property were studied:

a. Absorption and distribution by monitoring radioactivity in excreta and tissues at necropsy.

b. The potential for placental transfer and bioaccumulation in the fetus.

c. The disappearance rates from various body depots following a single parenteral injection.

3. BACKGROUND.

a. N,N-Diethyl-m-toluamide is the Army's standard insect repellent. It is intended for use as a solution in ethanol and as an aerosol for direct application to the skin and/or clothing.

b. Reregistration and classification procedures promulgated by Federal regulatory agencies have necessitated the reevaluation of m-Det to clarify certain toxicological aspects heretofore uninvestigated or lacking validation. This study addresses the dermal penetration of m-Det, its distribution in the body, and potential for accumulation in the unborn fetus.

Use of trademarked names does not imply endorsement by the US Army, but is intended only to assist in identification of a specific product.

4. MATERIALS.

a. Radiolabeled (ring ^{14}C) m-Det was obtained from New England Nuclear (NEN), Boston, MA. Reported specific activity was 13.6 millicuries (mCi)/millimole (mM) and total mass of 73.36 mg in 4.0 mL of methanol. The radiochemical purity was 98 percent as determined by radiochromatogram from a thin layer chromatography (TLC) plate using Silica Gel G and solvent system of Hexane:Ether:Acetic Acid (70:30:1 v/v/v). The compound contained a total radioactivity of 5.24 mCi and was identified as lot No. 1136-074; assay No. 78-147733. All dilutions of the material for administration to animals were prepared in absolute ethanol.

b. Unlabeled m-Det used in the teratology study contained a minimum meta-isomer content of 95 percent and maximum 5 percent of other isomers. The material was manufactured by Hardwicke Chemical Company, Elgin, SC, and packaged for McLaughlin Gormley King Company, 8810 Tenth Ave, Minneapolis, MN. The chemical was prepared as a 75 percent solution (w/v) in absolute ethanol. This solution was later spiked with ^{14}C-labeled m-Det for tracer registration in the animal.

5. ANIMALS. Absorption and distribution studies of ^{14}C m-Det were conducted using female New Zealand White rabbits, male and female Sprague-Dawley rats, and male Beagle dogs. Rabbits were purchased from Dutchland Laboratories, Inc., Denver, PA. Pregnant rabbits were purchased from the same source and received within 12 hours of breeding (day 0). Dogs were obtained from Hazelton Laboratories, Vienna, VA, and the rats were selected from US Army Environmental Hygiene Agency (USAEHA) breeding colonies. All animals were maintained on commercial chow and water ad libitum with a 12-hour, light-dark sequence. Ambient conditions were 24° $\pm$ 2°C and 45-55 percent relative humidity. Each animal was individually housed in a stainless steel metabolism cage throughout the study.

6. METHODS.

a. Single Intravenous (i.v.) Administration. Radiolabeled m-Det was first given intravenously to each species to determine the efficiency of the renal excretion process. Intravenous administration included injecting 5 microcuries (µCi) of the repellent into the marginal ear vein of the rabbit or 10 uCi into the cephalic vein of the dog. Rats were lightly anesthetized with ether and given 2 uCi of m-Det in the femoral vein. Appendix A presents the details of m-Det solution administration. Blood was drawn from dogs prior to injection and at timed intervals thereafter to document tracer disappearance from the circulation. Urine from each animal was collected

daily for 7 days, the volumes measured and normalized to a specific gravity of 1.024 grams per milliliter (g/mL). Aliquots (0.2 mL) of urine were then combined with 15 mL of PCS® II scintillation cocktail and radioactivity measured using a Beckman Liquid Scintillation Counter (Model LS200). Feces were also collected daily, weighed and homogenized in toto in about 5 volumes of water. Aliquots (0.2 mL) were then oxidized using a Harvey Biological Materials Oxidizer (Model LF 521) and the evolved $^{14}CO_2$ trapped in 15 mL of Oxifluor® - CO_2 scintillator. Blood specimens (0.2 mL) were similarly oxidized and radioactivity counted. Appropriate backgrounds and standards were run concurrently.

b. Single Topical Application. Topical application to each species was made to the clipped midlumbar region of the animal's back using a microliter syringe. The rate of application was always 4 micrograms per square centimeter ($\mu g/cm^2$). Each animal received the same radioactive dose as its i.v. counterpart; i.e., 2, 5, or 10 μCi. To maintain these parameters the area of application varied. The area was demarcated with petrolatum to contain the repellent while the solvent evaporated. Following topical application the area was covered with a nonocclusive patch[1] to prevent the contamination of excreta by the normal exfoliation of skin. The patch was changed at 24 hours and a new one affixed which remained throughout the test. Both patches were monitored following ethanol extraction for volatilized repellent trapped in the appliance. Urine and feces were collected daily for 7 days and radioactivity monitored as previously described.

c. Repeated Topical Application. Repeated applications of m-Det to pregnant rabbits throughout gestation were performed to assess the potential of the repellent for placental transfer and bioaccumulation in the fetus. Topical application to pregnant rabbits began on day 1 of pregnancy and daily thereafter through day 29 (1 day prior to expected parturition). Groups of four rabbits each were applied with 75-percent m-Det (w/v in ethanol) at levels of 50 milligrams per kilogram per day (mg/kg/day), 100 mg/kg/day, and 500 mg/kg/day. Sufficient radioactivity was added to the solutions such that each animal received about 0.5 μCi of radioactivity per day. Controls received only the ethanol diluent at volumes equivalent to the highest test group. Nonocclusive patches were monitored for volatilized radiocarbon at days 14 and 29. Urine specimens were collected daily and activity measured throughout the test. After the final urine collection (day 29) the animals were euthanized and tissue specimens collected. Fetuses were surgically excised and individually homogenized in about 2 volumes of water or methanol.

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Aliquots of water homogenates were oxidized to $^{14}CO_2$ while aliquots of methanol homogenates were combined with PCS and counted. A third method of documenting radioactivity in the fetus included homogenizing in water, then acidifying the suspension to pH 1.0 followed by repeated ether extractions. Aliquots of up to 1.0 mL of the extracts were combined with OCS®cocktail and activity measured.

d. Single Intravenous Injection to Pregnant Rabbits. To maximize the potential of ^{14}C-labeled m-Det to traverse the placental barrier, 12 pregnant rabbits in the 15th day of gestation were given a single i.v. dose of the repellent containing 15 µCi of radioactivity. At intervals of 1, 3, 6 and 24 hours after dosing, animals were euthanized and fetuses removed and oxidized in toto to measure radioactivity. Blood and tissue specimens from the parent were also collected and measured. Additionally, blood specimens were taken from rabbits surviving 24 hours, beginning immediately after injection and at timed intervals thereafter to document disappearance from the circulation.

e. Quantitation. The urinary and fecal excretion of ^{14}C following a single i.v. or percutaneous (p.c.) dose was quantitated over a 7-day study period. Excretion rates for each day's collection were calculated as percent of the initial injected or applied dose. Immediately following the final excreta collection, animals were sacrificed to determine the point of deposition of any retained labeled compound. Selected organs were excised and wet weighed intact. Representative specimens were taken from brain, lungs, liver, kidneys, spleen, testes, skin, muscle (thigh), fat (omental), bone (femur), and skin from the application site. One-fourth to one-half gram sections were oxidized to $^{14}CO_2$ and radioactivity counted. Calculations were based on counts/wet gram of tissue. Urinary excretion of the labeled moiety following repeated applications of m-Det to pregnant rabbits was calculated as percent recovery of the previous day's applied dose and later summarized as the mean daily absorption. Tissue specimens were also monitored.

7. RESULTS.

a. The percentage of ^{14}C-labeled m-Det recovered from animal urine following a single i.v. dose appears in Appendix B. The urinary pathway accounted for the largest quantitative elimination of injected m-Det, usually within the first 24 hours. Both male and female rats elicited the most rapid bioelimination, with 97 percent of the recovered radiocarbon appearing in urine within the first day. There was little apparent sex difference regarding elimination kinetics in this species. Rabbits and dogs showed similar excretion patterns though metabolic elimination was slower. Cumulative urinary excretion of m-Det through 7 days accounted for about 90 percent of the parenteral dose in rats and rabbits but was considerably less in the dog (52 percent). The repellent recovered in feces through 7 days measured less than 3 percent of the i.v. dose in each species. Tissue

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specimens monitored at necropsy were essentially void of measurable radioactivity (see Appendix C) as would be expected owing to the efficiency of the renal elimination process. Radiocarbon levels were always less than 1.0 parts per million (ppm) per gram of tissue.

b. Absorption of labeled m-Det as measured in urine following a single topical application appears in Appendix D. Penetration of the repellent occurred rapidly following topical application to each species with at least 75 percent of the absorbed dose appearing in the urine within the first day. Elimination was essentially complete at 3 to 4 days though absorption persisted throughout the 7-day test period as documented in the urine. The total percent recovery of ^{14}C m-Det following topical application to animals is presented in Appendix E and includes all parameters measured. The percent of absorbed compound is summarized for each species tested as the fraction of applied chemical appearing in the excreta through 7 days. The unabsorbed repellent was primarily recovered in the nonocclusive patches which initially trapped the evaporating compound and later, activity in exfoliated skin. Unabsorbed m-Det was also quantitated on or in the skin at the application site. Recovery of m-Det from tissue specimens at necropsy (see Appendix F) indicated the absence of or only trace amounts of the ^{14}C moiety in any tissue. No tissue, regardless of animal species, contained more than 1.0 parts per billion (ppb) of radiocarbon per gram. No affinity for specific tissue binding was noted.

c. Pregnant rabbits receiving 29 repeated applications of ^{14}C-labeled m-Det dermally reached a steady-state absorption/elimination pattern of about 45 percent of each day's dose, beginning in the first 24 hours and continuing throughout the test. The absorption mechanism was unaffected by the varying dose levels. Though a few of the test animals proved not to be pregnant, this too had little apparent effect on absorption through 29 days. Appendix G presents the cumulative recovery of m-Det in urine as well as unabsorbed fractions recovered at the application site and from the nonocclusive patches monitored at days 14 and 29. Cumulative absorption of the chemical by each rabbit accounted for about 45 percent of all compound applied. Unabsorbed compound accounted for an additional 35 percent, bringing the total accountable radioactivity for each animal to about 80 percent. This is in agreement with comparable values in rabbits following a single dermal application reported earlier. Tissue specimens analyzed for retained radioactivity in adult rabbits (see Appendix H) showed trace amounts of the ^{14}C moiety; these fractions usually appearing in specimens from organs having a high blood perfusion, i.e., liver, lung, spleen. No affinity for a specific target organ was observed nor could any trends be equated to dose level or state of pregnancy. Fetuses from pregnant rabbits treated daily (x29) with topical m-Det were monitored as earlier described. No detectable radioactivity above background ^{14}C levels was observed in any fetus analyzed, independent of methodology employed or dosage regimen. Analysis of blood taken at term from does also indicated the absence of circulating radiocarbon.

d. Pregnant rabbits receiving a single i.v. injection of labeled m-Det at day 15 of gestation and sacrificed at timed intervals thereafter portrayed the disappearance of the chemical from the blood, vital organs and fetuses. The summarized data appears in Appendix I and graphically in the Figure. The distributive phase of the compound in the animal body occurred within 1 hour of injection and elimination, though not a first-order process, rapidly followed. Tissue levels of m-Det correspond to levels in circulating blood throughout the first hour except the kidney which was markedly higher owing to its role in bioelimination. Fetuses registered the lowest radioactivity of any specimens monitored in comparable time frames, about six times lower than simultaneous blood levels recorded for the parent. Twenty-four hours after injection, elimination of the radiocarbon was all but complete. Detectable activity approached background levels in all specimens monitored. The lungs from tested animals were slower in clearing the m-Det and may implicate that organ's role in secondary bioelimination as $^{14}CO_2$. Blood specimens from rabbits measured periodically through 24 hours indicate a maximum distribution of m-Det in the body 15 minutes after injection. The calculated radiocarbon disappearance from circulating blood in the rabbit or half-life ($t_{1/2}$) measured 30 minutes. The comparable $t_{1/2}$ measured in dogs was 35 minutes. The reported[2] $t_{1/2}$ in man measured 240 minutes for the same compound.

8. DISCUSSION.

a. Ideally, an insect repellent possesses prolonged repellency towards a selected species of insect without appreciable absorption by the user. M-Det has long been the repellent of choice because of its low minimal effective dose (16 ug/cm^2)[3] yet may compromise the user because of its absorption potential. Smith, et al.[4] predicted about 50 percent absorption of m-Det following topical application to man. Spencer, et al.[5] in quantitating evaporation and penetration of the repellent to human skin in vitro reported similar findings. Feldman and Maibach,[2] however, demonstrated less than 9 percent absolute absorption of labeled m-Det in man following a 24-hour dermal exposure and 5-day collection period. In a similar test performed by Blomquist,[6] one adult human female demonstrated 5.5 and 3.8 percent absorption of topical m-Det in two separate 8-hour exposures and 48-hour collection periods. Had the two investigations followed like methodologies regarding exposure and collection periods, it is apparent that the values would have been nearly identical.

b. The collective animal data in the present study show substantial dermal penetration of ^{14}C-labeled m-Det as measured by urinary elimination. This pathway accounts for nearly all of the absorbed chemical, primarily in the first 24 hours after application. These observations are consistent with previously reported studies in animals[6,7] and man[2,6].

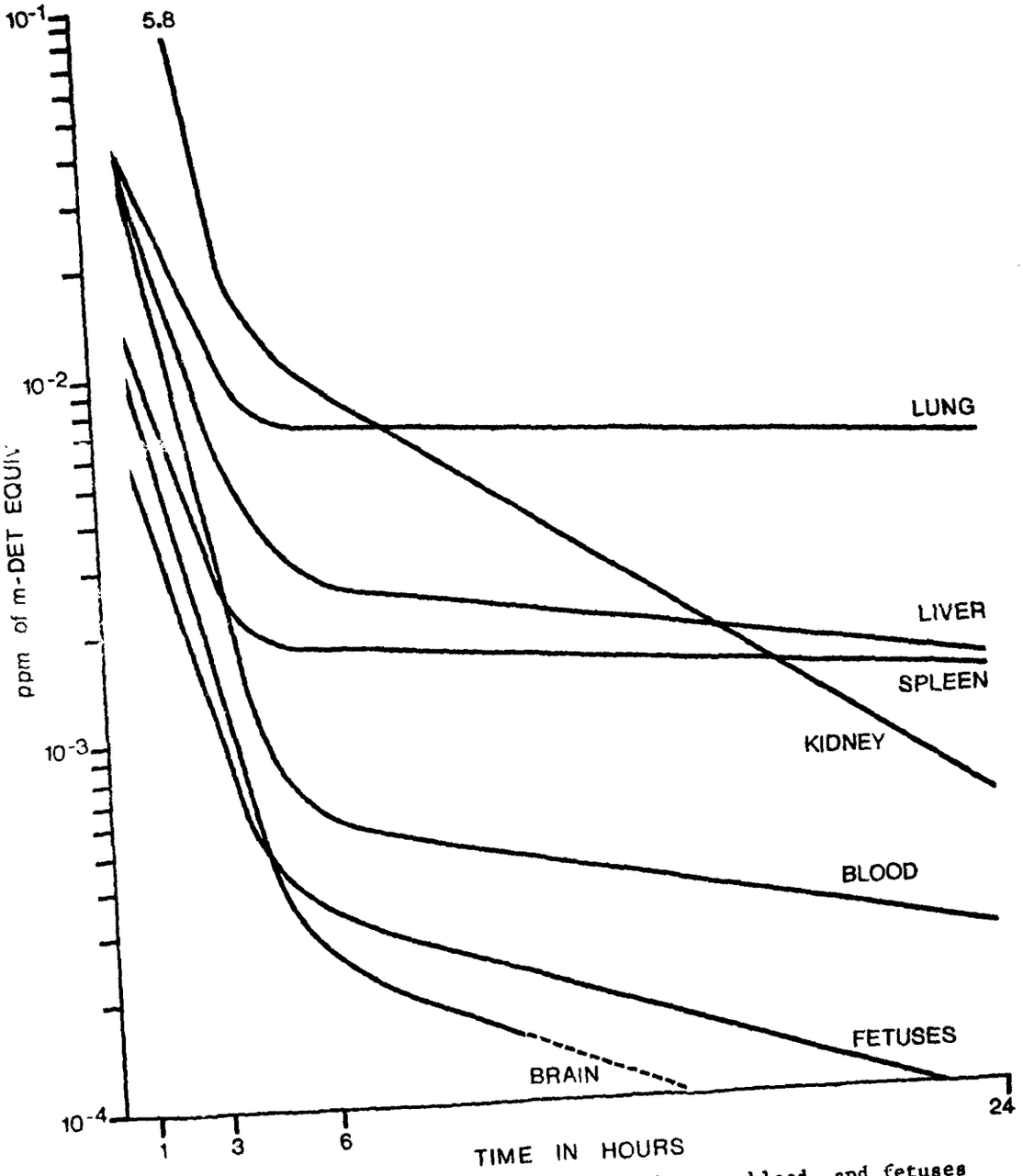

Figure - Disappearance of ^{14}C m-Det from tissues, blood, and fetuses following a single i.v. injection to pregnant rabbits at mid-term (15 µCi - 1.32 mg)

c. Fractional radioactivity persisted in the animals' urine through 7 days. Similar observations were made by Blomquist[6] using autoradiography procedures to register absorption of labeled m-Det in mice. In that test, persistent elimination, though small, was noted for 1 month after cutaneous application. Autoradiograms of mice 36 days after application showed a high radiocarbon content on or in the skin suggesting a "depot" effect in the epidermis and subsequent slow release into the circulation.

d. Enterohepatic elimination, though consistent in all animals tested, produced only marginal amounts of radioactivity as monitored in feces.

e. Although expired CO_2 was not monitored here, no significant radioactivity was detected by this portal in mice following a single parenteral dose of m-Det.[8]

f. Evaporation of the compound from the skin surface as measured by radioactivity trapped in the foam nonocclusive patch accounted for about one-third of the applied dose for each species. When coupled with unabsorbed chemical remaining at the application site 1 week later, the values (33-44 percent) approximate Spencers[5] findings (48 percent) which measured evaporation and residue of ^{14}C m-Det from human skin in vivo. Our values, though lower, are probably due to greater absorption by the animal, hence leaving less available for evaporation.

g. The dog showed the lowest absorption potential of the three species tested. This observation is consistent with previous studies[9] in this laboratory and adds further validity to the use of this mammalian species in predicting dermatotoxic hazards to man. To wit, a comparable study in man documented urinary excretion of labeled m-Det following i.v. or p.c. administration. Feldman[2], in the human test, reported 52 percent elimination of the i.v. chemical over a 5-day period, which is nearly identical to figures demonstrated in dogs. This indicates a remarkably similar metabolic treatment and bioelimination of the compound once it reaches the systemic circulation. Further similarities are observed following topical application. While comparisons between dog and man are at variance based on total absorption (31 vs 9 percent), the absorption/elimination kinetics correlate well. For example, if the fractional percent of total urinary elimination is compared on a day-to-day basis, the values for the first 3 days measure 76 vs 78 percent, 13 vs 12 percent, and 4 vs 5 percent, respectively, for dog and man. The differences in total m-Det absorption between the dog (and other animal species) and man, particularly in the first 24 hours, can in part be explained by anatomical differences in the integument. Scheuplein[10] observed that appendageal diffusion of topically treated human skin in vitro was dominant in the initial transient stage of diffusion, lasting perhaps 2 to 3 hours, followed by the more steady-state diffusion from the stratum corneum. If this analogy is related to the animal epidermis which contains far greater numbers of hair follicles per cm^2 and

more complex duct networks, one would expect greater diffusion through these appendegeal shunts during the initial transient phase. Beyond that, as the more steady-state absorption predominates, the integument differences between animal and man are partially reconciled and the comparative thicknesses of stratum corneum remain as the diffusion limiting factor. Scheuplein[10] further observed that the polar alcohols tend to diffuse through follicles with relative ease. While the absolute ethanol used in the animal tests (and certain commercial preparations) was allowed to evaporate, nearly 15 minutes evolved before this occurred, thus favoring a maximal penetration of the repellent during the first hour after application. The solvent factor should be considered in human-use formulations.

h. There appears to be little difference in distribution and elimination of m-Det following either parenteral or topical administration. Although numerical comparisons necessitate this assumption, Blomquist[6,8] also found no visual distribution differences in whole body autoradiograms of mice comparing the two routes of administration. It is likely that the chemical traverses the dermal barrier without appreciable biotransformation and upon reaching the systemic circulation is actively metabolized, probably in the liver, into metabolites readily eliminated in the urine. Schmidt[7] demonstrated the absence of intact m-Det in the urine of topically treated guinea pigs using isooctane extracts and chemical methods of analyses.

[illegible] specimens collected 1 week after parenteral or topical application to all species registered only trace radioactivity, usually only 1 or 2 counts above background levels. Similar observations were made by Blomquist[6,8] who noted no visual internal radioactivity in mice 4 days after i.v. m-Det injection or 6 days following cutaneous application.

[illegible]epeated applications of ^{14}C-labeled m-Det to pregnant rabbits at [illegible]s maximizing human use estimates failed to elicit a preponderance of the chemical for the conceptus. As noted earlier, no radioactivity in any [illegible]erm fetus registered higher than comparable sham-treated controls. This is at variance with observations made by Gleyberman, et. al.,[11] who reported accumulation of diethyl toluamide in fetuses (and maternal parents) following repeated dermal applications to rats. The methodology (not documented by Gleyberman) and species differences may partly explain the divergent findings, but neither the present study nor the autoradiographic investigations of Blomquist[6] using mice could substantiate these observations.

k. The ratio of absorbed m-Det to mass applied was not greatly affected by the various dose regimens employed in rabbits. Only a limited increase in cumulative absorption (42 vs 36 percent) was observed in animals repeatedly dosed at 50 mg/kg/day compared to others receiving a single-low dose of 70.3 µg of repellent. Although the comparison is made between pregnant and nonpregnant rabbits, it implies that the diffusion rate limiting barrier within the epidermis remains effective for the various doses employed and that the individual body burden should be proportional to the absolute mass of chemical applied.

l. Rabbits receiving a single intravenous dose of labeled m-Det at day 15 of pregnancy showed a rapid elimination of the repellent within 24 hours. Radioactivity in fetuses was always lower than corresponding maternal blood levels, decidedly lower than any maternal tissue except brain. This finding is consistent with an autoradiographic evaluation[8] in mice (advanced pregnancy) parenterally dosed with ^{14}C m-Det. By 4 hours after injection, the mouse fetuses contained very little radioactivity and no accumulation in any separate fetal organ could be distinguished.

m. At day 15 of gestation, histiotrophic nutrition of the conceptus should be minimal,[12] suggesting that a limited blood exchange of the chemical occurred between parent and fetus, probably by a passive diffusion process. This process is apparently reversible. As the radiocarbon level of maternal [illegible] declined with time, so also did the activity within the fetus. It is doubtful that the chemical would be metabolized by the rabbit fetus at this stage of maturation. To the contrary, the human fetus beyond the first trimester possesses drug-metabolizing enzymes whose rates of metabolism may approach 30 to 50 percent[12] of those of human adults. The human fetal kidney is also functional in eliminating metabolites. This may or may not be [illegible] to the unborn depending on the activity of individual metabolites, [illegible] renal excretion into the amniotic fluid presents itself for ingestion or reabsorption by the conceptus. Caution must therefore be exercised in predicting the effect of metabolized chemicals, particularly in the human fetus without knowledge of the potential toxic action of endogenous metabolites. Nonetheless, based on this investigation and others, no evidence for the bioaccumulation of the labeled moiety of m-Det in the animal fetus has been demonstrated. The absence of m-Det in the animal fetus has been demonstrated. The absence of embryotoxic/teratogenic anomalies in [illegible] progeny has been further documented[13] in this laboratory following repeated application of unlabeled m-Det, thus providing additional support for the original observations.

9. SUMMARY AND CONCLUSIONS.

a. The percutaneous penetration of ^{14}C-labeled m-Det was measured in three animal species following a single application. Absorption was assessed by monitoring excreted radioactivity in urine and feces daily for 7 days and analysis of representative tissue specimens at the end of the test period. Urinary excretion was the major elimination pathway of absorbed chemical in each species ranging between 30 percent for dogs and 43 percent for male rats. Enterohepatic elimination usually measured less than 1 percent. Nearly all of the unabsorbed repellent was recovered from a nonocclusive patch which protected the area and apparently trapped the evaporating compound. Measurable amounts of the chemical remained on or in the skin after 7 days. Tissue specimens from various body depots monitored 1 week after application showed only trace amounts or the absence of radioactivity, indicating little affinity of the chemical for tissue binding.

b. A concurrent study assessed the absorption of radiolabeled m-Det following repeated dermal applications to pregnant rabbits throughout the gestation period at dose levels maximizing human-use estimates. Progeny of the rabbits were also monitored to evaluate the potential of m-Det for placental transfer and bioaccumulation in the fetus. A steady-state elimination of absorbed repellent was reached within 24 hours of the first application which persisted throughout the study. No measurable [illegible] was detected in fetuses taken at term, independent of dose level or methodology employed.

c. A related test measured the disappearance of m-Det from various body depots at timed intervals following a single intravenous injection to pregnant rabbits at day 15 of gestation. Maximum radiocarbon blood levels were reached within 15 minutes and rapidly declined by a first-order process through the next 3 hours. Disappearance from the blood then slowed but approached background levels by 24 hours. Radioactivity in major organs followed a similar rate of disappearance. Radiocarbon levels in fetuses were detected at 1 hour after dosing but were always markedly lower than comparable maternal blood values. No potential for bioaccumulation in the conceptus was observed.

d. It is concluded that m-Det when applied to the skin should not present a dermatotoxic hazard to man based on these investigations and others. Absorption of the repellent would be expected to be less than 10 percent of the applied dose. Primary metabolic elimination of the absorbed chemical should occur within the first 24 hours via urinary excretion and be essentially complete after 3 days. Fractional absorption could persist longer if the repellent is not removed from the skin. No evidence of bodily retention or pooling of the radioactive moiety has been demonstrated in these tests. Although limited placental transfer of the repellent has been noted, it was readily eliminated from the rabbit fetus and no preponderance for bioaccumulation was observed. This observation, however, is self-limiting and projections to human subjects must remain guarded.

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APPENDIX A

DETAIL OF M-DET SOLUTION ADMINISTRATION

Animal Species	Route	Administration: Total Volume	Total Activity	Total Mass m-Det	Skin Area and Rate	Concentration of Solution
RAT	i.v.	0.1 mL	2 µCi	28.1 µg	---	281 µg/mL (20 µCi/mL)
	p.c.	0.1 mL	2 µCi	28.1 µg	7.02 cm^2 @4 µg/cm^2	281 µg/mL (20 µCi/mL)
RABBIT	i.v.	0.1 mL	5 µCi	70.2 µg	---	702 µg/mL (50 µCi/mL)
	p.c.	0.3 mL	5 µCi	70.2 µg	17.55 cm^2 @4 µg/cm^2	234 µg/mL (16.66 µCi/mL)
DOG	i.v.	0.1 mL	10 µCi	140 µg	---	1404 µg/mL (100 µCi/mL)
	p.c.	0.5 mL	10 µCi	140 µg	35.00 cm^2 @4 µg/cm^2	281 µg/mL (20 µCi/mL)
PREGNANT RABBIT	i.v.	0.16 mL	15 µCi	211 µg	---	1319 µg/mL (94 µCi/mL)

For repeated p.c. applications to pregnant rabbits, 75 percent m-Det (w/v) in ethanol was prepared and radioactivity added to each dose level as follows:

Level	Vol ^{14}C m-Det	Vol 75% m-Det (q.s.)	Concentration
50 mg/kg	1.0 mL (94 µCi/mL)	50 mL	1.88 µCi/mL
100 mg/kg	1.0 mL	100 mL	0.94 µCi/mL
500 mg/kg	1.0 mL	500 mL	0.188 µCi/mL

Example: to a 4-kg rabbit, at 100 mg/kg, apply 0.533 mL of 0.94 µCi/mL (750 mg/mL) solution. Application equals 400 mg of m-Det at an activity of 0.5 µCi.

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APPENDIX B

PERCENTAGE OF URINARY EXCRETION OF ^{14}C-LABELED M-DET FOLLOWING INTRAVENOUS ADMINISTRATION

	DAY	1	2	3	4	5	6	7	TOTAL
RAT, male	$\bar{x}$	87.26	0.43	0.30	0.10	0.06	0.05	0.04	88.24
2 µCi, 28.12 µg	S.D.	5.98	0.10	0.13	0.02	0.02	0.03	0.02	5.94
RAT, female	$\bar{x}$	89.96	[illegible]	[illegible]	[illegible]	[illegible]	0.10	0.02	91.55
2 µCi, 28.12 µg	S.D.	1.46	[illegible]	[illegible]	[illegible]	0.05	0.07	0.01	0.69
RABBIT, female	$\bar{x}$	74.39	14.24	3.31	0.89	0.32	0.25	0.13	93.63
5 µCi, 70.31 µg	S.D.	16.66	16.28	4.53	[illegible]	0.42	0.21	0.12	5.89
DOG, male	$\bar{x}$	46.43	1.84	2.04	0.87	0.82	0.09	0.05	52.14
10 µCi, 140.6 µg	S.D.	15.46	1.52	1.82	1.03	1.24	0.08	0.07	12.33

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APPENDIX C

RADIOCARBON RESIDUE OF M-DET IN ANIMAL TISSUES 7 DAYS AFTER A SINGLE INTRAVENOUS INJECTION

Species	Tissue	Responses	Mean Tissue Residue, ppb of m-Det Equiv
RAT, female	liver	3/3	1.0
	lung	3/3	0.4
	spleen	3/3	1.6
	kidney	3/3	0.1
	[illegible]	1/3	0.1
	[illegible]	1/3	0.1
	skin	2/3	0.1
RAT, male	liver	3/3	0.7
	lung	3/3	0.7
	spleen	3/3	1.2
	kidney	1/3	0.1
	skin	2/3	0.1
RABBIT	liver	5/6	0.3
	lung	2/6	0.2
	spleen	3/6	0.4
	kidney	1/6	0.1
	skin	1/6	0.1
DOG	liver	3/3	1.9
	lung	2/3	0.6
	spleen	3/3	0.6
	kidney	1/3	0.4
	skin	1/3	0.7

APPENDIX D

PERCENTAGE OF URINARY EXCRETION OF ^{14}C-LABELED M-DET FOLLOWING PERCUTANEOUS ADMINISTRATION*

		DAY 1	2	3	4	5	6	7	TOTAL
RAT, male	$\bar{x}$	39.55	2.20	0.61	0.22	0.14	0.29	0.21	43.22
[illegible] μCi, 28.1[illegible] μg	S.D.	6.79	0.45	0.21	0.16	0.04	0.50	0.29	6.75
[illegible], female	$\bar{x}$	26.[illegible]	1.[illegible]	1.39	0.32	0.25	0.27	0.71	32.42
[illegible] μCi, 28.[illegible] μg	S.D.	7.96	0.37	0.14	0.22	0.25	0.35	0.00	8.51
[illegible], female	$\bar{x}$	26.52	4.94	2.17	1.04	0.65	0.43	0.35	36.10
[illegible] μCi, 70.2 μg	S.D.	9.33	1.27	1.03	0.55	0.18	0.25	0.23	10.66
Dog, male	$\bar{x}$	22.94	3.90	1.21	2.16	0.23	0.24	0.09	30.77
10 μCi, 140 μg	S.D.	5.99	2.70	0.30	2.03	0.16	0.22	0.04	5.43

* Applied at a rate of 4 μg/cm^2

APPENDIX E

TOTAL PERCENTAGE OF RECOVERY OF ^{14}C-LABELED M-DET FOLLOWING PERCUTANEOUS ADMINISTRATION

		Urine	Feces	24-Hour Patch	7-Day Patch	Skin App. Area	Total
RAT, male	$\bar{x}$	43.22	0.43	27.58	4.17	6.67	82.07
	S.D.	6.75	0.09	7.47	1.03	1.40	
RAT, female	$\bar{x}$	32.42	0.44	30.83	1.63	1.23	66.55
	S.D.	8.51	0.16	6.48	0.77	0.86	
RABBIT, female	$\bar{x}$	36.10	2.26	21.50	6.65	15.39	81.90
	S.D.	10.66	1.22	6.97	4.19	8.63	
DOG, male	$\bar{x}$	30.77	0.40	32.00	3.34	2.83	69.34
	S.D.	5.43	0.23	3.63	0.80	1.02	

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APPENDIX F

RADIOCARBON RESIDUE OF M-DET IN ANIMAL TISSUES 7 DAYS AFTER A SINGLE PERCUTANEOUS APPLICATION

Species	Tissue	Responses	Mean Tissue Residue, ppb of m-Det Equiv
RAT, female	liver	2/3	0.4
	lung	1/3	0.3
	kidney	1/3	0.4
	muscle	1/3	0.3
	skin	3/3	0.3
RAT, male		NONE	
RABBIT	liver	2/6	0.1
	lung	1/6	0.3
DOG	liver	3/3	0.5
	lung	1/3	0.8
	spleen	1/3	0.8
	kidney	1/3	0.5
	testes	1/3	0.3

APPENDIX G

PERCENT RECOVERY OF ^{14}C-LABELED M-DET FOLLOWING REPEATED DERMAL APPLICATIONS TO PREGNANT RABBITS

Animal No.	Level mg/kg/day	14 Day Patch	29 Day Patch	Skin Appl. Site	Urine	Total % Recovery
513	50	19.88	22.55	2.57	35.96	80.96
516	50	16.47	17.53	16.47	45.24	95.71
517	50	14.56	19.62	2.00	44.93	81.11
518	50	4.13	5.85	16.73	43.26	69.97
522	100	15.00	24.89	7.55	43.86	91.30
520	100	15.43	17.32	3.42	36.11	72.28
526	100	10.41	15.77	3.63	51.72	81.53
514	100	10.92	16.42	3.43	61.24	92.01
521	500	8.61	11.64	3.03	49.90	73.18
525	500	8.08	15.47	5.72	47.17	76.44
519	500	5.86	18.99	5.56	44.28	74.69
524	500	12.46	16.71	3.28	45.38	77.83

APPENDIX H

RADIOCARBON RESIDUE OF M-DET IN TISSUES FROM PREGNANT RABBITS AT TERM FOLLOWING REPEATED DERMAL APPLICATIONS (X29)

Animal No.	Level mg/kg/day	Total mg Applied	Tissue	Tissue Residue ppm of m-Det Equiv.
513	50	3992	liver	3.2
			lung	2.0
			skin	1.0
516	50	4106	liver	5.9
			kidney	1.9
517	50	3770	liver	0.8
			kidney	0.8
518	50	4082		<M.D.Q. (minimum detectable quantity)
520	100	11,370	liver	3.9
			spleen	1.6
			kidney	5.5
			skin	1.6
526	100	10,148	liver	1.6
			lung	6.4
			kidney	4.8
514	100	8851	liver	4.8
			spleen	1.6
			kidney	7.9
522	100	7475		<M.D.Q.
521	500	51,435	liver	19.9
			spleen	7.9
			kidney	30.3
			skin	7.9
524	500	42,728	kidney	10.3
			bone	19.9
525	500	54,090		<M.D.Q.
519	500	54,945		<M.D.Q.

APPENDIX I

RECOVERY OF ^{14}C M-DET IN RABBIT TISSUES, BLOOD, AND FETUSES FOLLOWING A SINGLE INTRAVENOUS INJECTION
15 µCi (1.322 mg) each

Time Post Injection	Tissue Level µCi/gm Liver	Lung	Spleen	Kidney	Brain	Blood Level µCi/ml	Fetuses µCi/gm
1 hour	0.00420	0.00450	0.00160	0.06600	0.00118	0.00440	0.00063 331 CPM
3 hours	0.00090	0.00150	0.00036	0.00470	0.00020	0.00046	0.00014 97 CPM
6 hours	0.00031	0.00078	0.00020	0.00107	0.00003	0.00007	0.00004 33 CPM
24 hours	0.00016	0.00076	0.00015	0.00007	< M.D.Q.	0.00003	<0.00001 7 CPM

BLOOD LEVELS (CPM)							
5' (min)	15'	30'	60'	120'	3 hours	6 hours	24 hours
2127	2443	1765	1183	343	139	26	9

APPENDIX J

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